PATENT

REMARKS

Claims 1, 3-9 and 22 are currently pending. Formal drawings are provided herein with this amendment and response and the specification has been amended to conform the specification to the drawings. Further, the specification has been amended to correct certain typographical and clerical errors as well as to add the necessary sequence identifiers.

The application has also been amended to add claims 23-33, which recite particular embodiments of the invention disclosed in the application as filed, but not previously recited. Support for new claims 23-26 can be found in the specification at, for example, page 85, line 33, and bridging to page 86, lines 1 and 2; page 119, lines 29-32; and page 120, lines 3-7, 15-18, and 22-26. Support for new claim 27 can be found at, for example, page 84, lines 4-20. Support for new dependent claims 28-30 can be found at, for example, page 109, lines 7, 8, and 30; page 114, lines 5-19; and page 121, lines 17-20. Support for new claim 31 can be found throughout the specification and in particular on, for example, page 28, lines 10-24; Figure 13; page 74, line 20; page 75, lines 16-20; and page 104 line 8 through to page 108, line 7. Support for new claims 32 and 33 can be found, for example, at page 116, line 25 through page 117, line 20, Figure 18, Figure 19, and the descriptions of Figures 18 and 19 found at pages 18 and 19.

In addition, claims 1, 3-5, 9, and 22 have been amended to further expedite prosecution of the application and to set forth the invention with greater particularity as described in detail below. All of the amendments presented herein are fully supported by the specification as filed and no new matter has been added to the application.

Claims 1, 3, 4, 5, and 9 have been amended to set forth the claimed invention with greater particularity as set forth below. Claim 1, has been amended to recite "in an amount effective to inhibit tumorigenesis by inhibiting hyperproliferation of a human tumor cell having high *Nr-CAM* expression." Support for this amendment can be found in the specification at, for example, page 79, lines 21-26. Because claim 1 as filed recites that inhibition of hyperproliferation is in "a tumor cell" and because SEQ ID NO:1 encodes human Nr-CAM, Applicants believe that the present amendments merely clarify that which would be

known to the skilled artisan. Therefore, Applicants believe that these amendments are not narrowing.

Claim 3, has been amended to recite "administering to the subject." In addition, claim 3 has been amended to substitute the phrase "method of inhibiting proliferation of a human cell expressing *Nr-CAM*" (emphasis added). To maintain antecedent basis for the term "cell," the claim has also been amended to recite "hybridizable in the cell." Further, to conform the terminology of claim 3 to the amendment made to claim 1, claim 3 has been amended to recite "inhibits *Nr-CAM* expression." Support for these amendments can be found throughout the specification and in particular at, for example, page 74, line 20; page 75, lines 8-12 and lines 16-20; page 79, lines 21-32 and bridging to page 80, lines 1-4; and page 104 line 8, through to page 108, line 7. Because the claimed invention relates to antisense directed to human *Nr-CAM* sequences, Applicants believe that the skilled artisan reading the claim in light of the specification would understand that the inhibition of proliferation is in a human cell expressing *Nr-CAM*. Therefore, this amendment merely clarifies that which would be known to the skilled artisan and is not narrowing.

Claims 4, 5, and 9 have been amended to conform certain terminology to that of amended claim 3. In dependent claim 4, the phrase "in which the disease or disorder is a malignancy" has been amended to read "in which the human tumor cell expressing *Nr-CAM* is involved with a malignancy." Claim 5 has been amended to change the dependency to claim 4 and has further been amended to recite the disease is a "malignancy." Finally, claim 9, dependent from claim 3, has also been amended by substituting the phrase "... in which the human cell expressing *Nr-CAM* is involved with a disease or disorder selected from the group ..." (emphasis added). Support for these amendments can be found at, for example, page 60, lines 13-18, and page 72, lines 16-18. Applicants believe that the amendments to independent claim 3 are not narrowing for the reasons stated above, and the amendments to those claims dependent on claim 3 merely conform the terminology to that of the independent claim. Therefore, Applicants believe that the amendments to claims 4, 5, and 9 are not narrowing.

PATENT

Rejections Under 35 U.S.C. §112, First Paragraph

Claims 1, 3-9, and 22 stand rejected under 35 U.S.C. §112, first paragraph, the Examiner believing the claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner asserts, with regard to the antisense nucleic acids corresponding to human *Nr-CAM* (SEQ ID NO:1), that "no examples of use *in vivo* are taught in the specification as filed." The Examiner further asserts that Applicants have not provided in the specification "the necessary sequence structure of other antisense which bind to [*Nr-CAM*] for the *in vivo* functions claimed" and that the skilled artisan would not be able to "readily visualize" such sequences "absent further specific sequence structure design criteria." The Examiner, stating that "specific, not general guidance, is what is needed," believes that the specification only provides guidance for "murine full length antisense to specific regions of mice" and that the specification does not teach how to design either antisense to human *Nr-CAM* or how to design other *Nr-CAM* antisense for the claimed functions.

Applicants initially respectfully note, as set forth *infra* in response to the Examiner's remarks regarding enablement and "implied *in vivo* use," that claim 1 has been amended to recite a "composition comprising a pharmaceutically acceptable carrier ...," without acquiescing to any rejection or remark of the Examiner and to further expedite prosecution of the application. In view of this amendment, Applicants believe that the Examiner's remarks regarding *in vivo* function are inapplicable and, therefore, that the rejection of claim 1 for written description under § 112, first paragraph, is rendered moot.

Applicants also must respectfully disagree with the Examiner's statements regarding the characterization of the specification. The Examiner believes that only "murine full-length antisense to specific regions of mice are taught by way of example" and that the specification teaches "no examples of use *in vivo* ...." Applicants respectfully direct the Examiner to, for example, beginning at page 58 wherein various *in vivo* uses of the compositions of the present invention are disclosed. In particular, therapeutic uses of antisense molecules are disclosed at pages 79 through 81. Specific embodiments of the methods

disclosed at pages 79 through 81 are provided in the examples, at for instance, pages 119 and 120, where an antisense construct administered to mice is described as corresponding to human Nr-CAM of SEQ ID NO:1 (pCMV1/3Nr-AS which comprises nucleotides corresponding to a portion of the human *Nr-CAM* gene). Further, at page 119, line 32, the example explicitly refers to "antisense hNr-CAM". Applicants also respectfully point out that the example describes a standard xenograft murine model wherein the human antisense molecules are tested for their effect, in this case, on human tumors, derived from human glioblastoma cells subcutaneously injected into the mice. Thus, the *in vivo* data provided do not demonstrate the effect of the antisense composition on mice or mouse DNA sequences, but rather demonstrate the inhibitory effect of human *Nr-CAM* antisense on human tumor growth while many of the issues relating to stability of the antisense molecule and ability to penetrate the cell membrane and other issues can also be studied.

In addition, other factors must also be considered in determining whether the specification demonstrates an applicant was in possession of the claimed invention. These include, among others "the level of skill and knowledge in the art," "functional characteristics alone or coupled with a known or disclosed correlation between structure and function," and the "method of making the invention." MPEP § 2163 at 2100-163 (emphasis added); *see also Enzo Biochem, Inc., v. Gen-Probe Incorporated*, 63 USPQ2d 1609, 1613 (Fed. Cir. 2002) (*en banc*). Thus, genus claims to nucleic acids based on hybridization properties can satisfy the written description requirement, and functional characteristics are particularly sufficient where the skilled artisan would know how to carry out the invention as described.

In the present case, in addition to the characteristics and properties of the antisense nucleic acids as described in the specification at for example pages 74 through 79, *e.g.*, hybridization properties, as well as the described length ("at least 15 nucleotides"), various sequence-related and other criteria for the design of antisense molecules for *in vivo* use were well-known in the art at the time of filing the instant specification. *See, e.g.*, reviews of antisense technology cited by the Examiner in making the present rejection. In Ma *et al.*, *Biotechnology Annual Review* 5:155-196, (2000), for example at pages 160-165, the characteristics provided include antisense length, "CpG" content, and chemical modifications that enhance binding affinity and nuclease resistance. In Agrawal and Kandimalla, *Molecular*

Medicine Today 6:72-81 (2000), for example at page 77, characteristics for regions of a gene to target or to avoid are discussed. These characteristics include, for example, targeting the translation initiation codon region of mRNA (to inhibit formation of the ribosomal translation complex), avoidance of "GGGG" and "CpG" sites; minimization of secondary structures by avoiding, *e.g.*, self-complementary sequences or palindromes. Further, in Green *et al.*, *J. Am. Coll. Surg.* 191:93-105 (2000), for example at page 97, phosphorothioate linkages are discussed for enhancing the stability of antisense molecules, and it is also disclosed to avoid G-quartets and CpGs. Moreover, with respect to *in vivo* delivery of the antisense molecules, Green *et al.* state at page 97, second column, that "in vivo delivery of [oligonucleotides] appears to be more efficient than that which occurs in vitro."

Therefore, the skilled artisan would have known at the time the present application was filed that selection of antisense for the invention as claimed involved design criteria including (a) avoidance of GGGG sequences or CpG sites; (b) avoidance of self-complementary sequences, palindromes, and other sequences that promote secondary structures; (c) complementarity to, for example, ribosomal binding site sequences; and (d) use of certain chemical modifications, such as, for example, phosphorothioate linkages for *in vivo* stability. In light of the characteristics and properties disclosed in the specification as well as the other design criteria known in the art, the skilled artisan would recognize and be able to carry out the claimed invention upon reading the specification as filed. In fact, Agrawal and Kandimalla state at page 80, first column that, "if proper design precautions and controls are used," antisense oligonucleotides for *in vivo* use "can in fact be as simple as complementary base recognition.".

Further, Applicants respectfully note that the Synopsis of Application of Written Description Guidelines provide that antisense molecules to a particular nucleotide sequence are adequately described where the specification discloses the nucleotide sequence "which defines and limits the structure of any effective antisense molecules," the functional characteristics of the claimed invention, and an assay for screening for the claimed antisense molecules. In the present case, the specification discloses that antisense molecules for use *in vivo* include those functionally active in inhibiting cell proliferation as demonstrated in, *e.g.*, *in vitro* assays or animal models (*see* specification at page 59, lines 18-22). The specification

further describes cell proliferation assays for tumor cells *in vitro* (*see, e.g.*, specification at page 110, lines 28-32, bridging to page 111, and page 114, lines 4-19), as well as a standard murine xenograft model for determining the function of antisense molecules on human tumors *in vivo* (*see, e.g.*, specification at page 118, lines 12-31; page 119; and page 120, lines 1-26). Applicants believe that, in light of these structural and functional criteria as well as the design criteria, discussed *supra*, which define the distinguishing characteristics for effective antisense molecules to the human *Nr-CAM* gene of SEQ ID NO:1, one of ordinary skill in the art would conclude that Applicants were in possession of the invention as claimed.

Therefore, a reasonable correlation between human *Nr-CAM* antisense nucleic acid structure and *in vivo* function has been provided by the specification as filed in view of the relevant art. Using the guidance provided in the specification as well as characteristics well known in the art and routine methods for making the antisense oligonucleotides, the skilled artisan would be able to identify human *Nr-CAM* antisense sequences as claimed without an unreasonable expectation of success. Accordingly, Applicants believe that the claims satisfy the written description requirement under 35 U.S.C. § 112, first paragraph, as set forth above. Therefore, the Examiner is respectfully requested to reconsider and withdraw the rejection.

Claims 1, 3-9 and 22 also stand rejected under 35 U.S.C. §112, first paragraph, the Examiner asserting that the specification, "while enabling for compositions comprising the specific antisense ... to Nr-CAM of SEQ ID NO:1 taught in the specification as filed and methods of administration of the disclosed antisense *in vitro* ... and via injection to the specific glioblastoma tumors taught by way of example in mice, does not reasonably provide enablement for any antisense molecule to Nr-CAM nor any method of use of such molecules in any whole organism for the methods of inhibition claimed *in vivo*."

The Examiner initially states that the claims broadly read on "administration of any antisense to inhibit Nr-CAM of SEQ ID NO:1 *in vivo* for the *in vivo* uses claimed." With respect to claim 1, which is a composition claim, the Examiner has stated that claims drawn to "pharmaceutical compositions" have "implied *in vivo* use" and that such implication would be removed if the claims were amended to recite "a composition comprising a pharmaceutically acceptable carrier ...."

While not acquiescing to the Examiner's rejection, but in order to further expedite the prosecution of the instant application, Applicants have amended claim 1 in accordance with the Examiner's suggestion. Claim 1 now reads "A composition for the inhibition of tumorigenesis comprising a pharmaceutical carrier and an antisense nucleic acid …". In addition, to maintain antecedent basis in dependent claim 22, claim 22 as been amended accordingly to read, "The composition of claim 1, wherein the composition is formulated as a liquid."

In view of the amendment to claim 1 as described above, Applicants believe the Examiner's rejection of claim 1 for enablement under 35 U.S.C. § 112, first paragraph, to be mooted.

The Examiner also states that the specification teaches "by way of example administration of pCMV1/3Nr-AS … to mice glioblastomas and reduction of the tumor volume to no tumor" and that the specification is enabling "for administration of these antisense to glioblastomas by way of injection." The Examiner does not believe that the disclosure correlates to the breadth of the claimed invention "for the treatment of any tumor cell, by any means of administration with any [antisense] molecule that inhibits Nr-CAM … function." In restating this rejection from the previous Official Action additional references have been cited regarding alleged unpredictability in the art.

Initially, by way of clarification and as stated previously above in response to the written description rejection, Applicants again respectfully note that the examples in the specification referred to by the Examiner relate to a standard xenograft murine tumor model system comprising the subcutaneous injection of <u>human</u> glioblastoma cells into mice. Further, the antisense constructs used in the examples were to <u>human</u> *Nr-CAM*. The claims as amended recite antisense nucleic acids to human *Nr-CAM* (*i.e.*, "hybridizable in a cell to at least a portion of an RNA transcript of a *Nr-CAM* gene of SEQ ID NO: 1 …").

Further, the claims do not reasonably read on antisense "for the treatment of <u>any</u> tumor cell" as alleged by the Examiner. Interpretation of the claims must be consistent with the specification in view of the knowledge and skill in the art. MPEP § 2111 at 2100-46 and -47. The specification relates, *inter alia*, to inhibition of Nr-CAM function and, with respect to

cell overproliferation, to inhibition of Nr-CAM function in cells with high *Nr-CAM* expression. Thus, the skilled artisan reading the specification would not reasonably interpret the claims to read on antisense for inhibition in "any tumor cell." Moreover, claim 1 recites "... in an amount effective to inhibit hyperproliferation of a tumor cell <u>having high *Nr-CAM* expression</u>" (emphasis added).

Applicants also note, as stated above and in order to further expedite prosecution of the application, claim 1 has been amended in accordance with the Examiner's suggestion to recite "A composition ... comprising a pharmaceutical carrier ...", thereby obviating the Examiner's argument relating to an implied required *in vivo* use of the compositions.

Further, Applicants must again respectfully disagree with the Examiner regarding the predictability of *in vivo* use for antisense molecules and conclusions regarding alleged "undue experimentation." Applicants note, as a general matter, that "[t]he determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art." *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). Thus, in addition to unpredictability of the art, various other factors must be considered, including, *inter alia*, the amount of experimentation necessary, the state of the prior art, the relative skill of those in the art, and the nature of the invention, among others. *Id.* Thus, predictability must be viewed in relation to balancing all of the factors.

In this regard, Applicants respectfully refer the Examiner to Ma *et al.* (cited by the Examiner), who state at page 156 that, in contrast to the difficulty of designing "specific therapeutic agents ... based on the amino acid sequence of proteins[,] ... as a class of drug, synthetic [oligonucleotide] design is based on one unifying concept of base pairing between complementary nucleic acid sequences and thus, their target specificity." Thus, the very nature of the antisense art, upon which the claimed invention is based, renders a high degree of predictability regarding the targeting of specific genes.

Further, Applicant's respectfully disagree with the Examiner's reliance on the statement on page 167 of Ma *et al.* that "to gain therapeutic advantage ..., ODNs must ... be resistant to degradation, internalize efficiently, hybridize in a sequence specific manner with

the target nucleic acid, display adequate bioavailability with a favorable pharmacokinetic profile and be nontoxic." Assuming, *arguendo*, the accuracy of the statement, the recited characteristics do not render the instant claims non-enabled. The enablement requirement is met where one skilled in the art would "be able to practice the invention, given the level of knowledge and skill in the art." MPEP § 2164.08 at 2100-186. Here, the state of the art is such that the various characteristics recited can be addressed by particular design criteria known to the skilled artisan and those design characteristics described in the specification as filed. In fact, it appears that the statement cited by the Examiner may be merely a list of characteristics that an antisense molecule must have to be clinically effective as the remainder of the section in Ma *et al.* goes on the discuss how the art has approached and overcome each of the potential issues to achieve the characteristics listed.

In addition, several of the other references cited by the Examiner further discuss solutions to the "problems" set forth by the Examiner that have been disclosed in the art. *See generally*, Ma *et al.* at 160-165; Agrawal and Kandimalla at page 77; Green *et al.* at page 97. Agrawal and Kandimalla (which has been cited by the Examiner) at page 80, first column, state that, "if proper design precautions and controls are used," antisense oligonucleotides for *in vivo* use "can in fact be as simple as complementary base recognition." Further, Green *et al.* (also cited by the Examiner) at page 97 state that "[m]any of the limitations to antisense studies ... have been relatively easily dealt with." For example, Green *et al.* state that the use of phosphorothioate linkages "dramatically enhances oligonucleotide resistance to cleavage by nucleases," non-specific effects can be addressed by avoiding specific sequences such as G-quartets and C-G pairs, the use of cationic lipids "facilitate the delivery of ODNs in tissue culture," and "in vivo delivery of ODNs appears to be more efficient than that which occurs in vitro." An applicant is not required to describe all clinically effective methods or compositions claimed in an application in order to meet the requirements of 35 U.S.C. § 112.

In the present case, Applicants have demonstrated i) that Nr-CAM is involved in tumorigenesis of certain cancers, ii) that the inhibition of over expression of Nr-CAM can reduce the proliferation and migration of certain tumor cells, and iii) that antisense molecules can be used to reduce the expression of Nr-CAM *in vitro* and *in vivo*. Applicants have provided two specific examples of methods for introducing anti-sense molecules into cells.

PATENT

These methods include introduction of antisense by recombinant retroviral transformation and injection of antisense hNr-CAM expressing plasmids mixed with liposomes into mice. Applicants also provide three anti-sense molecules 17 to 21 nucleotides in length that will hybridize with the 5' end the Nr-CAM gene designed in view of the characteristics discussed above that are expected to hybridize with mRNA encoding Nr-CAM and to reduce the expression of Nr-CAM.

Applicants also disagree with the Examiner regarding stability and penetrability of oligonucleotides. Applicants respectfully refer the Examiner to Applicants previous response dated May 23, 2002, and again note that, at the time of filing of the instant application, various methods were known in the art that address the issues of cellular uptake and stability of oligonucleotides *in vivo*, resulting in increased resistance to degradation as well as increased penetration into cells. *See, e.g.*, page 76, line 2 through page 77, line 23 of the specification as filed; Schwab *et al.*, *Ann. Oncol.* 5 Suppl. 4:55-58, 1994 (greater intracellular stability through association with polyalkylcyanoacrylate nanoparticles); Green *et al.* at 97 (phosphorothioate linkages to enhance stability); Agrawal and Kandimalla at pages 77-78 (mixed-backb*one oligonucleotides (MBOs) with improved specificity in vivo stability); Jen et al.,* Stem Cells 18:307-19 (2000), at pages 314-315 (G-quartets and relationship of chirality to stability) (reference cited by the Examiner).

Also, with respect to *in vivo* delivery of antisense molecules, Applicants again respectfully disagree with the Examiner's reliance on Flanagan (regarding distribution of oligonucleotides to solid tumors following intravenous injections). As indicated above, Green *et al.* state at page 97 that "*in vivo* delivery of ODNs appears to be more efficient than that which occurs in vitro[;] ... [p]hosphorothioate ODNs delivered intravenously or intraperitoneally to murine models of inflammation, organ transplantation, and tumor xenografts have shown potent antisense inhibition without the need for [a] delivery reagent." Similarly, Bennett *et al.* (cited by the Examiner) at page 24, first column, disclose that a delivery reagent is not required for *in vivo* effects of oligonucleotides, and that this observation generally applies to different oligonucleotides in different *in vivo* models. In addition, Applicants have provided in the specification as filed examples wherein anti-sense molecules are capable of entry into human tumor cells that overexpress Nr-CAM in a murine xenograph

model. Thus, with regard to issues of delivery of oligonucleotides for *in vivo* use, the references cited by the Examiner appear to be discussing the issue of whether the *in vivo* activity of the oligonucleotides can be further enhanced. *See* Chirila *et al.*, *Biomaterials* 23:321-342 (2002) (noting, at page 327, second column, that "AS ODNs can be delivered without any carrier and they still can display AS activity" and questioning the need for delivery systems) (reference cited by the Examiner).

In addition, Applicants again disagree with the Examiner's statements regarding unpredictability of antisense molecules with *in vivo* specificity. Ma *et al.* (at, *e.g.*, pages 183-184), with respect to the *in vivo* use of antisense molecules and commenting on examples relating to dopamine receptor inhibition, state that "in contrast to conventional pharmaceutical drugs that act as antagonist to the receptor [which] have been found to be nonselective and [] not readily developed[,] [a]ntisense ODNs ... can be readily designed if the sequence encoding the receptor is known ...." The ability of such "readily designed" antisense to differentially inhibit a specific receptor *in vivo*, unlike conventional antagonists which interact with multiple subtypes, demonstrates, as stated by Ma *et al.*, "the high specificity of antisense ODNs." *Id.* at 184.

Further, as to the Examiner's reliance on statements in Branch regarding (1) the ability to manipulate antisense specificity *in vitro* and (2) the identification of effective antisense by empirical screening, Applicants respectfully refer the Examiner to points raised in the response to the previous Office Action filed May 23, 2002. Briefly, Applicants again note that the skilled artisan would recognize that antisense candidates for intracellular hybridization can be evaluated before screening on cells by known methods for determining complementary nucleic acid sequences that will exhibit specific hybridization coupled with consideration of cellular temperature and ionic content, which can be mimicked by manipulation of *in vitro* hybridization temperature and ionic conditions. Also, Branch does *not* state that empirical methods cannot reliably determine effective antisense molecules from a given set of candidates. Empirical methods do not by themselves constitute undue experimentation, nor do these methods speak to the predictability of success; and Branch states that "effective antisense molecules are typically selected from 20-50 candidates." (See page 49, second column.) Applicants believe that the Examiner has not specifically addressed these points.

Also, while Jen *et al.* state that "many additional considerations must be taken into account in applying these strategies in living cells including mRNA selection, drug delivery and intracellular localization of the antisense agent," such considerations can be addressed by the skilled artisan and, thus, the level of predictability relating to *in vivo* specificity does not render the claims nonenabled under 35 U.S.C. § 112. Site selection can be addressed by various known sequence-related design criteria (*see, e.g.*, discussion above regarding written description; Ma *et al.* at pages 160, 161 and 163; Agrawal and Kandimalla at page 77; Green *et al.* at page 97) and Green *et al.* at page 97 state that "in vivo delivery of [oligonucleotides] appears to be more efficient than that which occurs in vitro." Further, techniques were well-known in the art for the assessment of intracellular localization of antisense oligonucleotides. *See, e.g.*, Flanagan *et al.*

Applicants also disagree with the Examiner assertion that the claims are not enabled because teachings within the specification are not predictive of "treatment effects via other routes of administration." First, with respect to the references cited by the Examiner, Applicants respectfully note that Fritz *et al.*, while listing requirements for an efficient and versatile drug carrier system, continues to describe cationic polystyrene nanoparticles as a drug carrier system for antisense oligonucleotides and states that "[c]onclusively, the investigations demonstrate that the [polymeric] latexes prepared … fulfill the requirements for a suitable model drug delivery system for oligonucleotides." In addition, regarding Chirila *et al.* and internalization of the antisense into the desired cell target, Applicants refer the Examiner to the discussion above regarding *in vivo* delivery systems and again note that Chirila *et al.* state that antisense oligonucleotides can be delivered without any carrier and still display *in vivo* activity.

Further, Applicants note that the presence of inoperative embodiments within the scope of a claim does not render a claim non-enabled where the skilled artisan "could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with the expenditure of no more effort than is normally required in the art." MPEP § 2164.08(b) at 2100-2188. Applicants also respectfully note that it was well-known that antisense oligonucleotides could be administered by various routes of administration, including, *e.g.*, intravenous, subcutaneous, intradermal, and direct infusion to the target tissue; and the distribution of antisense oligonucleotides administered *in vivo* have been extensively

examined. *See* Ma *et al.* at pages 169 through 170. Indeed, with regard to pharmacokinetic behavior, phosphorothioate ODNs "are the most thoroughly studied class of compounds." *Id.* at 170. Thus, the effect of various parameters on distribution were known to the artisan, including modifications, tissue type, size of the antisense molecule, and administered dose. *See generally, e.g.,* Ma *et al.* Moreover, the observation that *in vivo* delivery of oligonucleotides can be more efficient than *in vitro* delivery, *see supra*, has been observed in studies using routes of administration other than direct injection, including, *e.g.*, intravenous and intraperitoneal. *See, e.g.*, Green *et al.* at page 97, second column; Bennett *et al.* at page 24, first column. In addition, methods were known for the assessment of oligonucleotide distribution following administration. *See, e.g.,* Ma *et al.* at page 170 (discussing use of labeled ODNs to determine uptake kinetics). Hence, the skilled artisan would have been able to determine effective routes of administration based on the information provided in the specification, the relevant art, and known techniques. Because determination of routes of administration is routine in clinical studies, such determination would not constitute undue experimentation. Therefore, because the presence of inoperative embodiments within the scope of a claim does not render a claim non-enabled where the skilled artisan "could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with the expenditure of no more effort than is normally required in the art," MPEP § 2164.08(b) at 2100-2188, the claims are also enabled for administration of the claimed *Nr-CAM* antisense molecules.

Applicants also respectfully disagree with the Examiner's statement at page 10 of the Office Action regarding the "almost certain toxicity of administration of antisense to Nr-CAM (a gene expressed in many tissues *in vivo*) systemically" and furthermore believe that the Examiner has not set forth a sufficient basis for this statement. Applicants note that the Examiner has stated, citing Jen *et al.*, "virtually all [oligonucleotides tested in phase I/II trials] have been characterized by a lack of toxicity," and further note that such antisense oligonucleotides tested in the clinic include those targeted to widely expressed genes including bcl-2 as discussed by Jen *et al.* at page 315, column 2 and PKC-α, as discussed by Green *et al.* at page 99, second column. In addition, assuming, *arguendo*, non-specific effects of Nr-CAM antisense administration *in vivo*, and as noted in Applicants' response dated May 23, 2002 to

the previous Office Action, side effects are routinely tolerated in the clinic and, given the nature of clinical studies and the reasonable expectations of time and other resources typically needed for determining appropriate dosing of a pharmaceutical composition, determination of dosage to avoid toxicity is a routine part of clinical development and, therefore, is not undue.

Moreover, Applicants strongly disagree with the Examiner regarding an alleged lack of correlation "with whole organism success in other organisms such as human." In this regard, Applicants initially note that the scope of enablement need "only bear a '*reasonable correlation*' to the scope of the claims." MPEP § 2164.08 at 2100-2186 (emphasis added). Further, as noted above, the specification as filed demonstrates the *in vivo* efficacy of *Nr-CAM* antisense on ***human*** glioblastomas subcutaneously injected into mice. However, the Examiner's arguments regarding lack of correlation appear to based on the notion that the glioblastomas were not heterologous to the mice studied. Because the glioblastoma cells in the tumors were human, there is a reasonable correlation with success of *in vivo* use on tumors in human subjects.

In addition, Applicants respectfully disagree with the Examiner's reliance on Resor *et al.* and Blackshear *et al.* with regard to the predictability of the models used. The Examiner relies on Resor *et al.* as stating that "transgenic approaches do not always and accurately reflect human carcinogenesis." Resor *et al.,* addresses transgenic models and is not applicable in the present claims because the animal model specifically disclosed in the application was not transgenic, but rather, as indicated above, was a nude mouse model using a human tumor cell xenograft. Further, Blackshear *et al.* is cited as stating "animal models of spontaneous and chemically induced mammary gland carcinogenesis ... do not faithfully mimic the pathology or biological behavior of human breast cancer ...." However, because the glioblastoma cells used were derived from a human glioblastoma tumor and thus were not "spontaneously or chemically induced," Blackshear *et al.* is also not relevant to the present case.

Accordingly, Applicants believe that the claims satisfy the enablement requirement under 35 U.S.C. § 112, first paragraph, as set forth above, and therefore in light of the above remarks and amendments, it is respectfully requested that the Examiner reconsider

and withdraw the rejection of claims 1, 3-9, and 22 for enablement under 35 U.S.C. § 112, first paragraph.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-467-9600.

Respectfully submitted,

Dated: 28 January 2003

Brian W. Poor

Brian W. Poor
Reg. No. 32,928

Customer No. 20350

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (206) 467-9600
Fax: (415) 576-0300
BWP/NVS/acg

SE 5014232 v6

APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Kindly replace the paragraph beginning at page 3, line 5, with the following substitute paragraph:

Glioblastoma multiforme are high grade astrocytomas that grow very rapidly and contain cells that are very malignant (Thapar and Laws, 1993, CA Cancer J. Clin., 43:263-271). The molecular basis of glioblastoma multiforme occurrence may involve systematic events at the chromosomal level or at a gene expression level. These may include inactivation of tumor suppressor genes, activation of oncogenes or specific translocations at the chromosomal level. Some genetic changes at the chromosomal level and gene expression level have been well documented for other brain tumors (Furnari *et al.*, 1995, Cancer Surv., 25:233-275). For example, it has been documented that loss of tumor suppressor(s) genes at chromosome 10, mutations in p53, or overexpression of epidermal growth factor receptor, may be major events leading to glioblastoma multiforme. A number of other genes such as EGFR, CD44, β4 integrins, membrane-type metalloproteinase (MT-MMP), p21, p16, p15, myc, and VEGF have been shown to be overexpressed in different types of brain tumors (Faillot *et al.*, 1996, Neurosurgery, 39:478-483; Eibl *et al.*, 1995, J. Neurooncol., 26:165-170; Previtali *et al.*, 1996, Neuropathol. Exp. Neurol. 55:456-465; Yamamoto *et al.*, 1996, Cancer Res., 56:384-392; Jung *et al.*, 1995, Oncogene, 11:2021-2028; Tsuzuki *et al.*, 1996, Cancer, 78:287-293; Chen *et al.*, 1995, Nature Med., 1:638-643; Takano, *et al.*, 1996, Cancer Res., 56:2185-2190; Bogler *et al.*, 1995, Glia, 15:308-327). Several cell adhesion molecules (CAMs), such as integrins, cadherins, IgSF proteins (carcinoembryonic antigen, N-CAM and VCAM-1) or lectins, are thought to be involved in tumorigenesis (Johnson, 1991, Cancer Metastat. Rev. 10: 11-22). Over-expression of anti-sense to the secreted glycoprotein ~~SPRAC~~ **SPARC** (secreted protein, acidic and rich in cysteine), results in suppression of the adhesive and invasive capacities of melanomas (Ledda *et al.*, 1997, Nature (Med). 3: 171-176). The cell-surface adhesion molecule MCAM (MUC18) when over-expressed may lead to increased adhesion and metastatic potential of human melanoma cells in nude mice (Xie *et al.*, 1997, Proc. Nat'l

Cancer Conf. 38:522). Expression of N-CAM or ICAM (~~Intracellular~~ **Intercellular** adhesion molecule) is related inversely to increased metastasis (Hortsch, 1996, Neuron 17:587-593). Other genes such as p53 show mutations in the majority of brain tumors (Bogler *et al., supra*). How the interplay of one or more of these genes leads to tumorigenesis is not known but most likely multiple steps are required for neoplastic transformation. The exact series of events that lead to initiation or progression of glioblastoma are not known at present and useful markers for early detection of brain tumors are lacking.

Kindly replace the paragraph beginning at page 6, line 5 with the following substitute paragraph:

DCC is a cell adhesion molecule that belongs to the N-CAM family. DCC was first shown to be expressed in a variety of tumors including the brain and lung but its expression was reduced and mutated in a number of colorectal carcinomas (Fearon, *et al.*, 1990, Cell, 61:759-767). The ~~down regulation~~ **down-regulation** or mutation of the DCC molecule ~~lead~~ **leads** to the disruption of normal cell-cell adhesion in the intestinal epithelium. This process is known to play an important role in the metastasis of colorectal carcinomas (Albelda, 1993; Fearon *et al.,* 1990).

Kindly replace the paragraph beginning at page 13, line 22 with the following substitute paragraph:

Figures 2 (A-D) present the nucleotide and amino acid sequences of human Nr-CAM ~~has~~ as well as the results of nucleotide sequence analysis as described in Section 6 (Figure 2C) and a schematic illustration of the *hNr-CAM* gene showing the area used herein for antisense targeting (Figure 2D). Figure 2A presents the nucleotide sequence of human Nr-CAM **(SEQ ID NO: 1)** . Features of the nucleotide sequence include the following: Nucleotides 130-3615 encode the extracellular domain; nucleotides 202-4026 encode product = hBRAVO-Nr-CAM; nucleotides 316-483 encode the Immunoglobulin I domain; nucleotides 613-768 encode the Immunoglobulin II domain; nucleotides 988-1134 encode the Immunoglobulin III domain; nucleotides 1258-1410 encode the Immunoglobulin IV domain;

nucleotides 1540-1719 encode the Immunoglobulin VI domain; nucleotides 2113-2265 encode the first Fibronectin (Fn) repeat; nucleotides 2413-2565, the second Fn repeat; nucleotides 2710-2886, the third Fn repeat; nucleotides 3028-3186 the fourth Fn repeat; nucleotides 3370-3510, the fifth Fn repeat; nucleotides 2616-3684, the transmembrane region; nucleotides 3685-4036, the intracellular domain; and nucleotides 4030-4134 constitute a 3' untranslated region. Figure 2B presents the amino acid sequence of human Nr-CAM **(SEQ ID NO: 2)**. The hydrophobic signal sequence is underlined. Figures 2A and 2B are adapted from Lane *et al.*, 1996, Genomics 35:456-465.

Kindly replace the paragraph beginning at page 14, line 13 with the following substitute paragraph:

Figure 2C illustrates nucleotide sequence identity analysis between previously cloned *hNr-CAM* (Accession Number U55258**; SEQ ID NO: 3**), *rat Nr-CAM* (Accession Number U81037**; SEQ ID NO: 4**) and the sequence of clone D4-1 **(SEQ ID NO: 5)** obtained by cloning the hNr-CAM isolated by DD-PCR into pCRII vector (Invitrogen). Sequence identity analysis was performed using the DNasis program from Hitachi Software (South San Francisco, CA). Stars (*) indicate presence of identical nucleotides among the sequences.

Kindly replace the paragraph beginning at page 20, line 19 with the following substitute paragraph:

Figure 24 shows the sequence identity analysis between human **(SEQ ID NO: 31)** and rat **(SEQ ID NO: 32)** *Nr-CAM* nucleotide sequence.

Kindly replace the paragraph beginning at page 36, line 18 with the following substitute paragraph:

The *Nr-CAM* sequences provided by the present invention include those nucleotide sequences encoding substantially the same amino acid sequences as found in native Nr-CAM proteins, and those encoded amino acid sequences with functionally equivalent amino acids, as well as those encoding other Nr-CAM derivatives or analogs, as described in Section ~~5.2.2~~ **5.2.5** *infra* for Nr-CAM derivatives and analogs.

Kindly replace the paragraph beginning at page 54, line 16 with the following substitute paragraph:

In addition, assays that can be used to detect or measure the ability to inhibit, or alternatively promote, cell proliferation are described ~~in Section 5.4~~ **herein**.

Kindly replace the paragraph beginning at page 63, line 5 with the following substitute paragraph:

The Therapeutics of the invention that antagonize Nr-CAM activity can also be administered to treat ~~,~~ **or** inhibit premalignant conditions and to inhibit or prevent progression to a neoplastic or malignant state, including but not limited to those disorders listed in Table 1. Such prophylactic or therapeutic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see Robbins and Angell, 1976, Basic Pathology, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-79). Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Kindly replace the paragraph beginning at page 72, line 10 with the following substitute paragraph:

Additional methods that can be adapted for use to deliver a nucleic acid encoding a Nr-CAM protein or functional derivative thereof are described ~~in Section 5.5.2.2.2., *infra*~~ **herein below**.

Kindly replace the paragraph beginning at page 73, line 32 with the following substitute paragraph:

In other embodiments, chemical mutagenesis, or homologous recombination with an insertionally inactivated *Nr-CAM* gene (see Capecchi, 1989, Science 244:1288-1292 and Section 5.14 *infra*) can be carried out to reduce or destroy endogenous Nr-CAM function, in order to decrease cell proliferation. Suitable methods, modes of administration , and compositions ~~,~~ that can be used to inhibit Nr-CAM function are described ~~in Sections 5.8.2 through 5.8.2.1.2 above~~ **herein**.

Kindly replace the paragraph beginning at page 80, line 27 with the following substitute paragraph:

Additional methods that can be adapted for use to deliver a Nr-CAM antisense nucleic acid are described ~~in Section 5.9.1.4~~ **herein**.

Kindly replace the paragraph beginning at page 98, line 4 with the following substitute paragraph:

In this study, the role of Nr-CAM ~~,~~ in brain tumorigenesis was characterized.

Kindly replace the paragraph beginning at page 108, line 10 with the following substitute paragraph:

Genomic Southern blot was performed as described in Section 6.1.7 on 3 brain tumor cell lines (astrocytoma III, glioma and glioblastoma) and the NIH3T3 cell line. As show in ~~Figures~~ **Figure** 9 ~~(A and B),~~ no change in the genetic level of *hNR-CAM* was observed in the 4 cell lines tested.

Kindly replace the paragraph beginning at page 118, line 6, with the following substitute paragraph:

As shown in Figure ~~20~~ **20C**, a 17 fold increase in the number of cells undergoing apoptosis was observed. These results clearly suggest that antisense hNr-CAM over-expression caused 5GB glioblastoma cells to become more sensitive to UV radiation.

Kindly replace the title of "TABLE 4" at page 119, line 9, with the following substitute title:

THE EFFECT OF ANTISENSE [*NNR-CAM*] ***hNr-CAM***
EXPRESSION ON TUMOR FORMATION *IN VIVO*

Kindly replace the paragraph beginning at page 128, line 29 with the following substitute paragraph:

In order to identify genes that are altered by the *hNr-Cam* gene product in 5GB glioblastoma cells, we compared the expression of 5000 genes in pCMV-neo or pCMV-1/3Nr-AS transfected 5GB glioblastoma cells using the Array technique. Two identical Human GeneFilters™ were differentially hybridized with cDNA prepared from pCMV-neo or pCMV-1/3Nr-AS transfected 5GB glioblastoma cells. Two identical array membranes containing 5000 genes were [purchase dfrom] **purchased from** [REsearch] **Research** Genetics. The membranes were prehybridized in a pre-hybridization solution for 12 hours. Hybridization was done with a [1x105] **$1x10^5$** cpm/ml cDNA probe. This probe was prepared by carrying out 1st strand synthesis from pCMV-neo or pCMV-1/3Nr-AS transfected 5GB glioblastoma cells 1μg [polyA+mRNA] **$polyA^+$ mRNA**. First strand cDNA synthesis was carried out using the Advantage cDNA synthesis kit from Clontech. The membranes were washed in a wash solution (0.1%SDS/1XSSC) for 30 minutes at room temperature and then at 50°. Membranes were then exposed to X-ray film. Results are presented in Figures 26 (A and B).

IN THE CLAIMS:

1. (Twice amended) A ~~pharmaceutical~~ composition for the inhibition of tumorigenesis comprising **a pharmaceutical carrier and** an antisense nucleic acid comprising at least 15 nucleotides hybridizable in a cell to at least a portion of an RNA transcript of a *Nr-CAM* gene of SEQ ID NO: 1 in an amount effective to inhibit **tumorigenesis by inhibiting** hyperproliferation of a **human** tumor cell having high *Nr-CAM* expression.

3. (Twice amended) A method of inhibiting ~~cell over~~ proliferation **of a human cell expressing *Nr-CAM*** in a subject comprising administering to ~~a tumor in a~~ **the** subject ~~in which such treatment or prevention is desired~~ an effective amount of a *Nr-CAM* antisense nucleic acid comprising at least 15 nucleotides that inhibits *Nr-CAM* ~~function~~ **expression**, wherein the *Nr-CAM* antisense nucleic acid is hybridizable in ~~a~~ **the** cell to at least a portion of a RNA transcript of the *Nr-CAM* gene of SEQ. ID. NO.: 1.

4. (Amended) The method according to claim 3 in which the ~~disease or disorder~~ **human cell expressing *Nr-CAM*** is **involved with** a malignancy.

5. (Amended) The method according to claim **4** ~~3~~ in which the ~~disease or disorder~~ **malignancy** is selected from the group consisting of brain cancer, leukemia, and B cell lymphoma.

9. (Amended) The method according to claim 3 in which the **human cell expressing *Nr-CAM* is involved with a** disease or disorder [is] selected from the group consisting of premalignant conditions, benign tumors, hyperproliferative disorders, and benign dysproliferative disorders.

22. (Amended) The ~~pharmaceutical~~ composition of claim 1, wherein the ~~pharmaceutical~~ composition ~~comprises~~ **is formulated as** a liquid.